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Variations in Major Cations and Certain Heavy Metals in the Serum of the Blue Crab, Callinectes sapidus

James Alden Colvocoresses
College of William and Mary - Virginia Institute of Marine Science

VARIATIONS IN MAJOR CATIONS AND CERTAIN HEAVY METALS IN THE SERUM OF THE BLUE CRAB *Callinectes sapidus*

A Thesis

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James Alden Colvocoresses

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APPROVAL SHEET

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Author

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Maurice P. Lynch

Kenneth L. Webb

Paul A. Haefner, Jr.

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ABSTRACT

Concentrations of sodium, magnesium, calcium, potassium, copper, zinc, lead and cadmium were determined in serum samples taken from mature blue crabs, <u>Callinectes sapidus</u>, collected from a range of environmental conditions in Virginia waters and previously analyzed for other serum constituents including protein, chloride and osmotic concentration. Seasonal variations in constituents were studied by analysis of monthly samples collected from the York Spit area, lower Chesapeake Bay from Aug. '70 to July '71. A cyclic seasonal variation in mean sodium was found which paralleled that earlier reported for chloride and osmotic concentration, with highest means recorded for winter months (432-471 meq/l) and lowest for summer months (376-391 meq/l). Mean potassium also showed a cyclic pattern, but with highest values recorded for late summer (7.75-8.01 meq/l) and lowest for late winter (6.01-6.30 meq/l). Mean calcium and magnesium did not exhibit any obvious seasonal trends.

Salinity effects on serum constituent levels were studied in samples collected during the summer ('71) along the Lower Chesapeake Bay-York River transect (0.2 - 29.0 o/oo). Serum sodium and potassium exhibited a positive relationship with environmental salinity, while serum calcium and magnesium showed much greater variability and no clear relationship to salinity. Regulation of sodium followed the same pattern as previously reported for osmotic concentration, with the serum hyper-ionic to the medium below about 28 o/oo and isoionic above this point. Regulation of potassium resembled that found for chloride, with hyperionic concentrations below about 24 o/oo and hypoionic concentrations above that point. Calcium was maintained at hyperionic levels over the entire salinity range. Serum magnesium was maintained hypoionic to the environment at salinities over about 7 o/oo, and hyperionic below that point. Serum calcium was found to be correlated with total serum protein (r = .68).

Serum levels of copper and zinc were not found to be related to either salinity or temperature but were both found to be closely related to total serum protein. Zinc and calcium levels were found to be more closely related than copper and calcium levels despite the fact that copper was more closely related to protein than was zinc.

No detectable amounts of lead or cadmium were observed in the serum of any of the samples analyzed.

VARIATIONS IN MAJOR CATIONS AND CERTAIN HEAVY METALS

IN THE SERUM OF THE BLUE CRAB

Callinectes sapidus

INTRODUCTION

The blue crab, *Callinectes sapidus*, Rathbun, has been observed to exhibit wide fluctuations in population density from year to year (Pearson, 1948; Van Engel, 1958). The reasons for these fluctuations are as yet poorly understood. It has been suggested (Jeffries, 1964) that the use of chemical or physiological indices might prove valuable both in monitoring the condition and predicting the future abundance of these populations. Subsequent attempts to validate this approach have been hampered by a lack of knowledge relative to the normal variability of potential indicators both in relation to existing environmental conditions and seasonal variations (Jeffries, 1966).

Lynch (1972) performed a study designed to first supply the required information of normal or "baseline" values of several serum constituents and serum osmotic concentration in the blue crab and then related this information to values observed in naturally or experimentally stressed populations. The constituents studied included chloride, total protein, glucose, total ninhydrin positive substances and free amino acids. Baseline values were determined through extensive sampling of mature hard crabs (molt stages C_4 and C_4T, Passano,

1960) along several salinity gradients and through monthly collections made over several years in waters of relatively constant salinity. Natural stresses included red tide and pesticide induced kills; experimental stresses were induced by holding in experimental tanks and rapid increase in temperature.

The response of several of the constituents to the different types of stress was quite variable, with both the degree and direction of response for a given constituent depending upon the type of stress involved. This variability of response prompted the suggestion that the best indices for associating specific causes of stress with serum constituent levels will probably incorporate a combination or profile of several constituents. The specific entities which contribute to total serum protein and to osmotic concentration were indicated as the most promising serum constituents for further study towards this end.

The potential indicated by Lynch's work (1972) for the development of physiological indices based on serum constituent levels in the blue crab prompted the present study. Preserved, unused fractions of a substantial number of the serum samples collected and analyzed during his study were further analyzed to determine "baseline" serum levels of the four major cations sodium, magnesium, calcium and potassium, and the heavy metals copper, zinc, cadmium and lead.

SERUM CATIONS

Osmotic regulation in the blue crab has been rather extensively studied (Odum, 1953; Tan & Van Engel, 1966; Ballard & Abbott, 1969: Tagatz, 1971; Lynch et al., 1973), but ionic regulation by this animal has been only superficially considered. Serum cation analyses have only been performed on laboratory-held experimental animals. Tan (1962) studied the regulation of sodium and potassium; Mantel (1967) also studied sodium regulation; Haefner (1964) studied calcium regulation (though chiefly in respect to molting), but only Gifford (1962) has studied the regulation of all four major cations in the blue crab, and then only in relation to hypersaline stress.

Temperature was suggested as a major factor affecting osmoregulatory ability in the blue crab by Ballard & Abbott (1969). Lynch et al. (1973) confirmed seasonal temperature-related trends in serum osmotic concentration and serum chloride levels. Sodium, as the major cation in electrolytic balance with chloride, should also follow a similar trend, but the effects of temperature on the regulation of the three other cations have been previously unexamined in the blue crab and only sparsely studied for any decapod (Dehnel 1962, 1967; Dehnel & Carefoot, 1965).

Glynn (1968) found that serum calcium and protein levels maintained a fairly constant ratio during fluctuations associated with the molt cycle of *Homarus vulgaris*. This pattern of fluctuation was basically the same as had been found for molt-related changes in serum protein for decapods in general (Passano, 1960) and molt-related calcium fluctuations in the serum of the blue crab (Haefner, 1964). Whether intermolt decapods maintain related levels of serum protein and calcium had not previously been investigated.

SERUM COPPER AND ZINC

Copper and zinc are both known to be of considerable metabolic importance in decapod crustacea. Copper is the metallic oxygen carrier bound with hemocyanin, the respiratory pigment of the blue crab, and zinc is a known co-factor of carbonic anhydrase, the principle enzyme involved in calcification (Florkin, 1960). Serum copper and zinc levels have been reported for a substantial number of decapod species (Bryan, 1968) and considerable work has been reported concerning the metabolism of these metals in higher crustacea (Florkin, 1960; Bryan 1964, 1966, 1967; Djanghmah, 1970; Djanghmah and Grove, 1970).

Serum copper in the blue crab has been studied by Horn & Kerr (1963, 1969) but no information is available concerning zinc in this animal. Serum levels of both metals have been found to be strongly related to serum protein concentrations in the other decapod species thus far studied, a fact verified for copper in the blue crab by Horn & Kerr (1963). Bryan (1968) found that while most decapods maintained similar copper to blood solid ratios, the zinc to blood solid ratio varied from species to species. Hemocyanin and other blood proteins have been found to comprise nearly all of the total organic content of most crustacean sera (Florkin, 1960).

Lynch and Webb (1973) reported differences in serum protein levels with sex and year class as well as seasonal differences in the serum protein of female blue crabs. These differences should also apply to serum levels of copper and zinc if the aforementioned relationship to serum protein is sufficiently strong.

Copper and zinc have been found to be accumulated by crustacea to concentrations many times higher than those found in normal seawater. Serum concentrations of these metals have been reported to be well regulated and relatively independent of environmental concentrations (Bryan 1966, 1967; Djanghmah, 1970) under normal circumstances. Under conditions of extremely high environmental concentrations, these metals may inhibit respiration (Corner & Sparrow, 1956; Kerkut & Munday, 1962; Hubschmann, 1966), but these effects occur at concentrations so high (about 20 - 30 mg/l) that they could not reasonably be expected in an estuary except in the immediate vicinity of a severe pollution source of these metals.

SERUM CADMIUM AND LEAD

Virtually nothing is known of the metabolism of heavy metals other than copper and zinc in decapod crustacea (Bryan, 1971). No values could be found for cadmium and lead levels in any crustacean serum. The establishment of "baseline" serum values for these metals should provide a limited insight as to how they might be metabolized by the blue crab or other decapods as well as providing a basis for future comparisons.

MATERIALS AND METHODS

COLLECTION OF CRABS

The serum samples analysed in this study were collected in a two part program (Lynch et al., 1973). The first program consisted of year-round collection of crabs from a site of relatively constant salinity. The second program consisted of transect collections being taken along the salinity gradient found in Virginia.

The crabs used in the seasonal study were obtained from commercial crabbers, and had all been taken from the York Spit area of lower Chesapeake Bay. The crabs obtained were taken in crab pots except during winter (December-March), when crab dredges were used (Van Engel, 1962). Samples analyzed for this study were obtained monthly from August 1970 to July 1971, with the exception of May 1971.

Crabs used in the salinity gradient study were taken by trawling with a 30 foot semi-balloon trawl from R/V Pathfinder. These trawls were made during the summer of 1971 and covered the salinity gradient found from the mouth of Chesapeake Bay to the head-waters of the York River. The samples used in the seasonal study were the same as those used by Lynch et al. (1973), while the salinity gradient samples used in the present study were obtained in a later but similarly planned collection program. During April-June 1972, several spot collections were taken in pots to determine the effects of freezing and clot removal on the serum levels of the cations and metals studied.

ENVIRONMENTAL DATA

Water samples were collected by a variety of methods (bucket, Kemmerer bottle, Frautsche bottle). Temperatures were measured to the nearest 0.1° C. Salinities were determined to the nearest 0.01 o/oo with an Industrial Instruments Laboratory salinometer (RS-7A). Environmental data were obtained simultaneously with trawling samples. Environmental data to accompany the samples acquired from commercial crabbers were obtained during the monthly trawl surveys conducted by the Virginia Institute of Marine Science.

BLOOD SERUM

Crab blood was obtained by cutting through the merus of one of the rear walking legs and collecting the dripping blood in a heavy-walled test tube or a centrifuge tube. The blood samples were cooled (by refrigeration or placing in crushed ice) prior to further processing. In the laboratory, the clot which was normally present was broken up with a glass stirring rod and the blood centrifuged in an International (PRC-2) centrifuge at 2,000 g for 25 minutes at 5° C. The expressed serum was pipetted from the clot and stored in a polyethylene capped vial. The samples were then frozen until analyses could be performed. Analyses were done on serum samples from each individual. Experiments run in conjunction with the present study with freshly collected blood samples verified that freezing, clot removal and storage in polyethylene produced no significant changes in either serum cation or metal levels.

MORPHOMETRIC MEASUREMENTS AND LIFE STAGE DATA

At the time of bleeding the long width, *i.e.*, the distance between lateral spine tips, was measured to the nearest millimeter. At the same time sex, maturity, year class (if females) and state of the molt cycle were determined. Sex and maturity were determined using the criteria of Van Engel (1958), i.e., mature females had a broad rounded abdomen free of the ventral shell while immature females had a triangular abdomen sealed to the body. Mature males had a T-shaped abdomen either hanging free from the ventral shell or held in place against this shell by a pair of "snap-fastener-like tubercles". Immature males had a T-shaped abdomen sealed tightly to the ventral shell.

The year class of mature females was determined using the criteria described by Hopkins (1947). Earlier year class crabs (older) which had already spawned, possessed adult-sized nemerteans (*Carcinomertes sp.*) on the gills and usually eroded or fouled carapaces. Later year class (younger) crabs generally had bright, unfouled carapaces and no, or only very small nemerteans on the gills.

SERUM ANALYSES

All of the samples used in the present study had been previously analyzed for total osmotic concentration, chloride and total serum protein by the methods described in Lynch *et al*. (1973) and Lynch and Webb (1973).

Serum levels of sodium, magnesium, calcium, potassium, copper, zinc, cadmium and lead were determined by atomic absorption spectroscopy. Samples were prepared for analyses by diluting 0.5 ml of serum to 10 ml with deionized distilled water, adding 1 drop conc. HNO_3, and centrifuging at 2,000 g for 15 min. The supernatant was then analyzed directly for Zn, Cd, and Pb. Determinations of serum levels of Cu and the cations studied, with the exception of Na, were made on samples which had been further diluted to the optimum concentration range of the most sensitive line.

Na was determined by using the 3302 A line recommended for concentrated solutions (Environment Protection Agency, 1971). Lanthanum chloride was added to the Ca and Mg samples and standards to prevent anionic interferance. The sodium content of the potassium standards was approximately matched to the sample concentrations to avoid sodium enhancement of the potassium values.

The serum samples taken in the seasonal study were analyzed on a Varian Techtron AA-5 unit, those from the salinity gradient study were analyzed on a Beckman Atomic Absorption System-DB-G Spectrophotometer module using a Varian W18G100 Burner. Duplicate analysis on the two machines indicated no differences greater than reproducibility on a single unit.

STATISTICAL ANALYSIS

Differences between mean values of the various constituents in the samples were considered significant if "t" fell outside the 95% confidence interval of a two tailed distribution ($P<0.05$) when compared using the student "t" tests for small samples described by Snedecor (1956).

Correlation coefficients were calculated on a Monroe 1766 programable calculator using program 4022-s (Linear regression and correlation coefficient with test of significance of r). Significance was again ascribed on the same basis by comparing the "t" value calculated by the program to a two-tailed distribution.

Significant differences between correlation coefficients were determined using the method described by Snedecor (1956), with differences being considered significant if the calculated "t" value for a given pair of r's exceeded 2.807 ($P < .005$, $df = \infty$).

RESULTS

ENVIRONMENTAL DATA

In the seasonal study, salinity varied from 17.3 to 27.0 o/oo and temperature from 0.5 to 24.1° C. The respective ranges for the salinity gradient study were 0.2 to 29.0 o/oo and 19.5 to 29.3° C.

PREVIOUSLY DETERMINED CONSTITUENTS

Data on the seasonal variation of total osmotic concentration, serum chloride and total serum protein in these samples have been reported in Lynch _et al_. (1973) and Lynch & Webb (1973). The corresponding data for the salinity gradient serum samples used in this study are presented in Tables 1, 2 and 3.

SERUM SODIUM

In the seasonal study, mean serum sodium ranged from 376 to 471 meq/liter (Table 4). With the possible exception of the January sample, a distinct seasonal cyclical variation in serum sodium levels was found, with high values occuring during winter months and low values during the summer (Fig. 1). This variation was found to bear a strong negative correlation with temperature ($r = -.86$). Strong positive

Sample date	Area	Salinity (o/oo)	Temperature (°C)	Mean total serum osmotic concentration ± S.E. (milliosmoles) Males (N)	Females (N)
1971					
3 June	YR	14.42	20.7		794 (1)
3 June	YR	12.69	20.5	735 ± 12 (5)	722 (1)
3 June	YR	9.05	21.1	681 ± 19 (4)	691 ± 1 (2)
3 June	YR	5.09	21.7	679 ± 7 (4)	658 ± 19 (4)
7 June	YR	24.26	19.7	769 ± 9 (4)	807 ± 22 (3)
7 June	CB	26.34	19.5		792 ± 15 (3)
8 July	YR	19.60	24.6		718 ± 6 (3)
8 July	YR	16.72	25.6	712 (1)	688 ± 9 (4)
8 July	YR	13.52	26.5	704 ± 10 (2)	699 ± 12 (3)
8 July	PR	6.42	28.2		650 ± 9 (2)
8 July	PR	0.15	28.3	565 (1)	
12 July	CB	20.83	24.1		784 (1)
12 July	CB	28.97	21.5	845 (1)	817 ± 11 (2)
4 August	YR	21.63	26.1	731 ± 6 (4)	755 ± 11 (2)
4 August	YR	19.90	27.5	782 (1)	
4 August	YR	17.40	27.6	710 ± 4 (4)	739 ± 6 (4)
4 August	YR	14.29	28.7	721 ± 7 (4)	715 ± 12 (4)
4 August	PR	9.30	28.9	712 ± 13 (4)	
4 August	PR	3.72	29.3	705 ± 9 (2)	
9 August	YR	21.46	26.5	760 ± 9 (5)	773 ± 27 (2)
9 August	CB	24.85	25.7	790 ± 15 (2)	811 ± 7 (3)

Area code: YR, York River; CB, Chesapeake Bay; PR, Pamunkey River.
*Significant difference.

Table 1 - Mean serum osmotic concentration in mature, hard blue crabs, *Callinectes sapidus*, collected from various salinities in Virginia.

Sample date	Area	Salinity (o/oo)	Temperature (°C)	Mean serum chloride ± S.E. (meq/liter)	
				Males (N)	Females (N)
1971					
3 June	YR	14.42	20.7		330 (1)
3 June	YR	12.69	20.5	321 ± 5 (5)	332 (1)
3 June	YR	9.05	21.1	323 ± 6 (4)	327 ± 5 (2)
3 June	YR	5.09	21.7	320 ± 12 (4)	315 ± 6 (4)
7 June	YR	24.26	19.7	373 ± 3 (4)	361 ± 10 (3)
7 June	CB	26.34	19.5		380 ± 18 (3)
8 July	YR	19.60	24.6		354 ± 9 (3)
8 July	YR	16.72	25.6	333 (1)	338 ± 6 (4)
8 July	YR	13.52	26.5	318 ± 6 (2)	325 ± 4 (3)
8 July	PR	6.42	28.2		312 ± 5 (2)
8 July	PR	0.15	28.3	252 (1)	
12 July	CB	20.88	24.1		337 (1)
12 July	CB	28.97	21.5	340 (1)	383 ± 8 (2)
4 August	YR	21.63	26.1	370 ± 12 (4)	425 ± 5 (2)
4 August	YR	19.90	27.5	390 (1)	
4 August	YR	17.40	27.6	365 ± 4 (4)	378 ± 3 (4)
4 August	YR	14.29	28.7	353 ± 5 (4)	368 ± 5 (4)
4 August	PR	9.30	28.9	350 ± 4 (4)	
4 August	PR	3.72	29.3	340 ± 10 (2)	
9 August	YR	21.46	26.5	392 ± 9 (5)	386 ± 11 (2)
9 August	CB	24.85	25.7	404 ± 9 (2)	389 ± 7 (3)

Area code: YR, York River; CB, Chesapeake Bay; PR, Pamunkey River.
*Significant difference.

Table 2 - Mean serum chloride concentration in mature, hard blue crabs, *Callinectes sapidus*, collected from various salinities in Virginia.

Sample date	Area	Salinity (o/oo)	Temperature (°C)	Mean serum protein ± S.E. (mg/ml) Males (N)	Females (N)
1971					
3 June	YR	14.42	20.7		106.27 (1)
3 June	YR	12.69	20.5	59.62 ± 15.51 (5)	36.09 (1)
3 June	YR	9.05	21.1	34.22 ± 12.75 (4)	25.24 ± 0.96 (2)
3 June	YR	5.09	21.7	48.02 ± 4.66 (4)	28.67 ± 15.23 (4)
7 June	YR	24.26	19.7	25.99 ± 2.91 (4)	70.99 ± 29.23 (3)
7 June	CB	26.34	19.5		53.81 ± 12.07 (3)
8 July	YR	19.60	24.6		20.81 ± 4.92 (3)
8 July	YR	16.72	25.6	17.72 (1)	23.25 ± 3.62 (4)
8 July	YR	13.52	26.5	35.43 ± 0.97 (2)	52.38 ± 14.60 (3)
8 July	PR	6.42	28.2		25.10 ± 5.56 (2)
8 July	PR	0.15	28.3	40.12 (1)	
12 July	CB	20.88	24.1		75.81 (1)
12 July	CB	28.97	21.5	94.53 (1)	66.50 ± 9.55 (2)
4 August	YR	21.63	26.1	36.28 ± 8.91 (4)	47.26 ± 15.38 (4)
4 August	YR	19.90	27.5	19.84 (1)	
4 August	YR	17.40	27.6	22.65 ± 2.90 (4)	36.42 ± 10.43 (4)
4 August	YR	14.29	28.7	33.31 ± 8.40 (4)	41.94 ± 15.67 (4)
4 August	PR	9.30	28.9	54.58 ± 20.42 (4)	
4 August	PR	3.72	29.3	62.39 ± 13.59 (2)	
9 August	YR	21.46	26.5	20.05 ± 5.45 (5)	40.08 ± 24.45 (2)
9 August	CB	24.85	25.7	50.09 ± 6.06 (2)	49.47 ± 5.73 (3)

Area code: YR, York River; CB, Chesapeake Bay; PR, Pamunkey River.
*Significant difference.

Table 3 - Mean total serum protein concentration in mature hard blue crabs, _Callinectes sapidus_, collected from various salinities in Virginia.

Sample date	Salinity (o/oo)	Temperature (^{o}C)	Mean serum sodium ± S.E. (meq/liter)		
			Males (N)	Old females (N)	New females (N)
1970				1968 class	1969 class
18 August	27.01	24.1		359 ± 39 (2)	380 ± 10 (11)
23 September	24.06	24.1	352 (1)	383 ± 19 (4)	379 ± 6 (8)
20 October	23.50	21.6		379 ± 42 (3)	414 ± 16 (10)
12 November	22.48	14.9	408 ± 15 (2)		430 ± 17 (11)
8 December	22.02	7.6	478 (1)		442 ± 20 (12)
1971					
21 January	19.94	4.8	471 (1)	402 ± 16 (2)	434 ± 21 (10)
19 February	22.34	0.5		489 ± 69 (2)	468 ± 13 (11)
17 March	17.28	6.2			424 ± 12 (13)
				1969 class	1970 class
13 April	21.22	8.8	410 ± 32 (5)	404 ± 10 (3)	
2 June	24.26	19.7	448 (1)	388 ± 13 (10)	359 (1)
21 July	21.31	24.1	381 ± 9 (8)		363 ± 19 (3)

Table 4 - Seasonal variation of mean serum sodium concentration in mature hard blue crabs, *Callinectes sapidus*, from the York Spit area, Chesapeake Bay, Virginia.

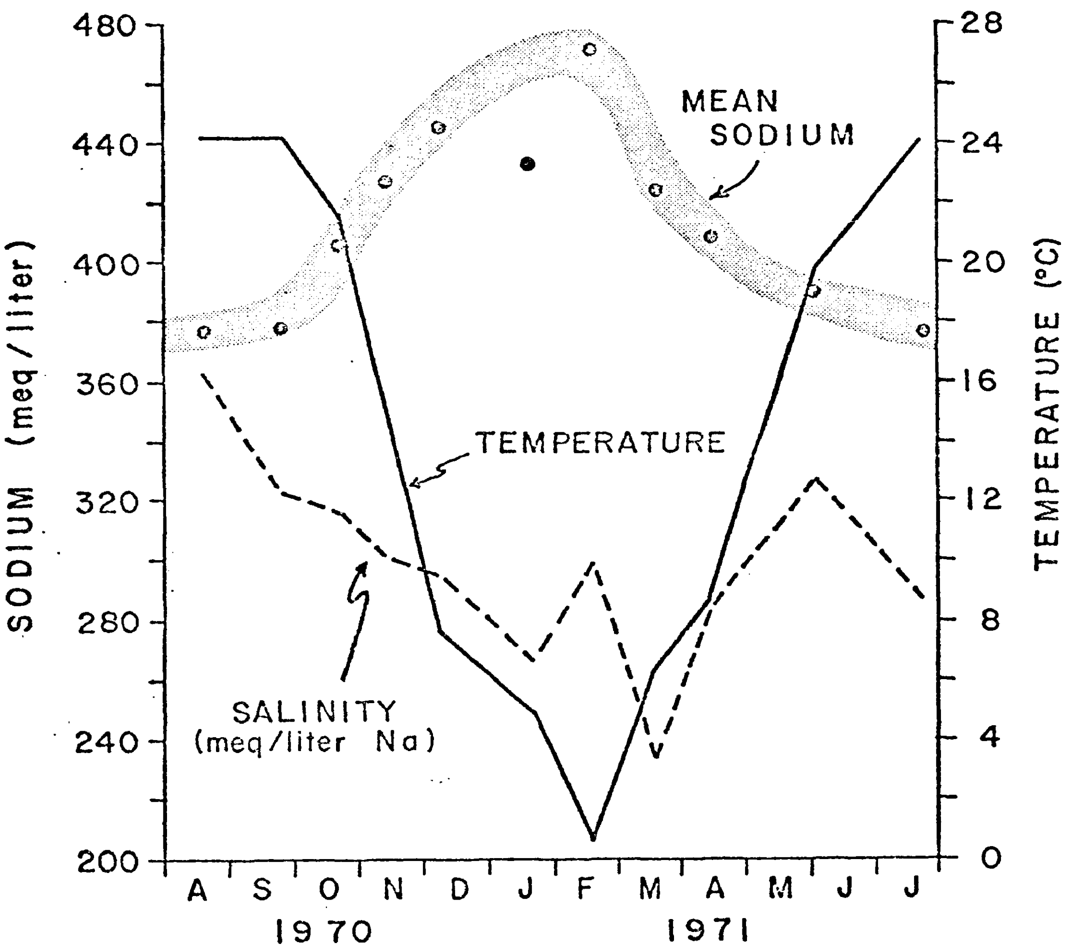

Figure 1 - Seasonal variation of mean serum sodium concentration in mature, hard blue crabs, <u>Callinectes sapidus</u>, from the York Spit area, Chesapeake Bay, Virginia.

correlations were found between serum sodium and serum osmotic concentration (r = .94), and between serum sodium and serum chloride (r = .88). No significant differences were found between mean serum sodium levels of males and females taken in the same sample, or between serum sodium levels of different year class females.

In the salinity gradient study, mean serum sodium ranged from 331 to 415 meq/liter (Table 5). Serum sodium levels are maintained hyperionic to the environment from fresh water up to about 26-30 o/oo, where they approach isotonicity (Fig. 2). No significant sexual differences were found. A positive correlation was found between serum sodium and salinity (r = .80).

SERUM POTASSIUM

In the seasonal study, mean serum potassium ranged from 6.01 to 8.01 meq/liter (Table 6). Serum potassium levels remained relatively high and constant during summer and fall, fell sharply with the onset of winter, and recovered gradually during the spring (Fig. 3). The positive correlation with temperature (r = .68) was significantly less than the negative relationship between sodium and temperature. A significant negative correlation was found between serum potassium and serum chloride (r = -.59). No

Sample date	Area	Salinity (o/oo)	Temperature (°C)	Mean serum sodium ± S.E. (meq/liter) Males (N)	Females (N)
1971					
3 June	YR	14.42	20.7		353 (1)
3 June	YR	12.69	20.5	353 ± 7 (5)	376 (1)
3 June	YR	9.05	21.1	364 ± 15 (4)	353 ± 1 (2)
3 June	YR	5.09	21.7	347 ± 9 (4)	347 ± 15 (4)
7 June	YR	24.26	19.7	394 ± 14 (4)	396 ± 10 (3)
7 June	CB	26.34	19.5		392 ± 3 (3)
8 July	YR	19.60	24.6		387 ± 17 (3)
8 July	YR	16.72	25.6	333 (1)	372 ± 4 (4)
8 July	YR	13.52	26.5	375 ± 3 (2)	356 ± 11 (3)
8 July	PR	6.42	28.2		353 ± 18 (2)
8 July	PR	0.15	28.3	315 (1)	
12 July	CB	20.88	24.1		383 (1)
12 July	CB	28.97	21.5	397 (1)	415 ± 7 (2)
4 August	YR	21.63	26.1	349 ± 8 (4)	357 ± 5 (2)
4 August	YR	19.90	27.5	350 (1)	
4 August	YR	17.40	27.6	357 ± 3 (4)	347 ± 3 (4)
4 August	YR	14.29	28.7	349 ± 11 (4)	340 ± 10 (4)
4 August	PR	9.30	28.9	331 ± 9 (4)	
4 August	PR	3.72	29.3	330 ± 17 (2)	
9 August	YR	21.46	26.5	399 ± 9 (5)	399 ± 9 (2)
9 August	CB	24.85	25.7	394 ± 0 (2)	406 ± 15 (3)

Area code: YR, York River; CB, Chesapeake Bay; PR, Pamunkey River.
*Significant difference.

Table 5 - Mean serum sodium concentration in mature, hard blue crabs, *Callinectes sapidus*, collected from various salinities in Virginia.

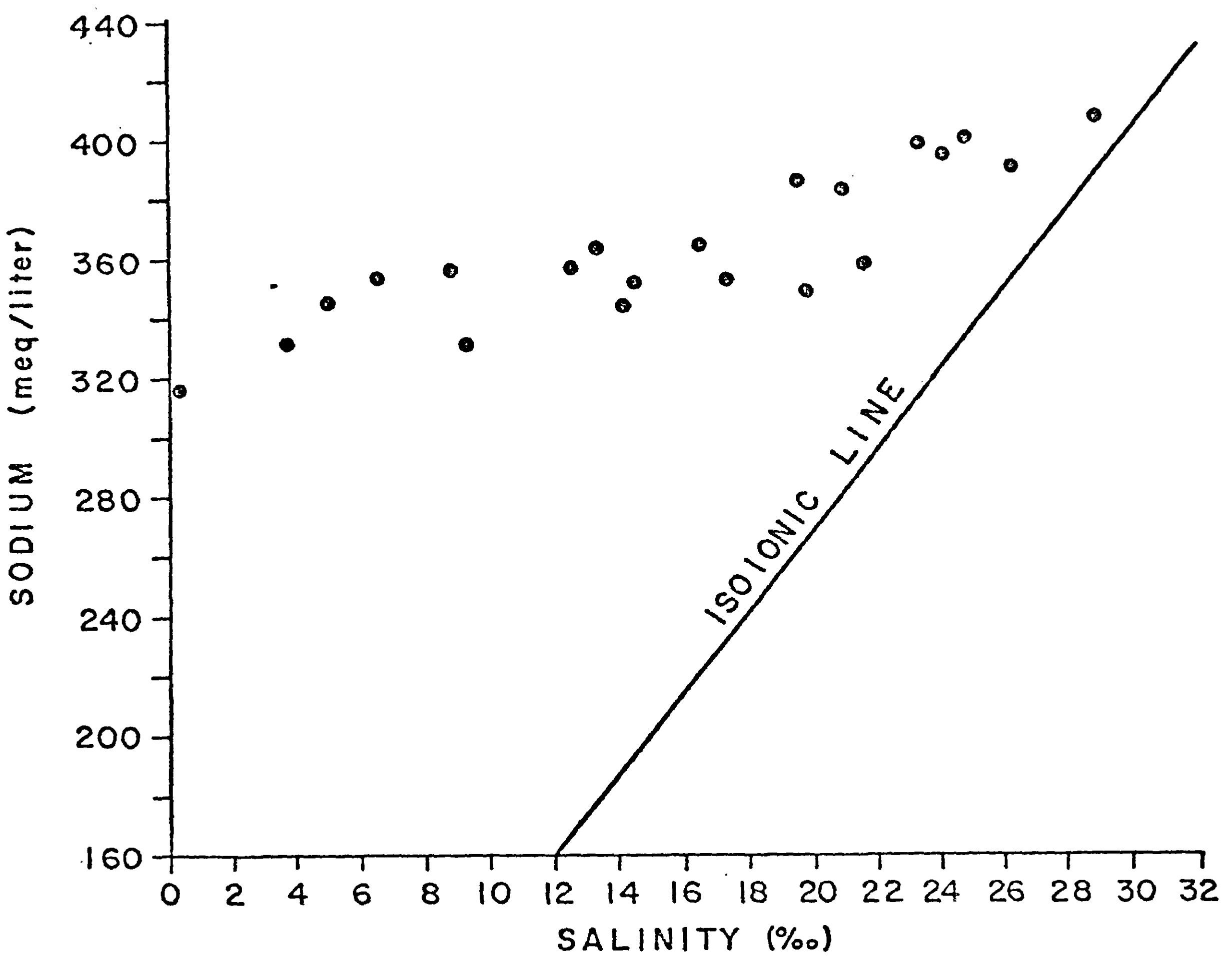

Figure 2 - Mean serum sodium concentration in mature, hard blue crabs, Callinectes sapidus, collected from various salinities in Virginia.

Sample date	Salinity (o/oo)	Temperature (°C)	Mean serum potassium ± S.E. (meq/liter) Males (N)	Old females (N)	New females (N)
1970				1968 class	1969 class
18 August	27.01	24.1		7.94 ± 1.32 (2)	7.78 ± 0.40 (11)
23 September	24.06	24.1	5.51 (1)	8.26 ± 0.79 (4)	7.95 ± 0.34 (8)
20 October	23.50	21.6		6.97 ± 0.52 (3)	7.57 ± 0.55 (10)
12 November	22.48	14.9	6.86 ± 0.72 (2)		7.86 ± 0.44 (11)
8 December	22.02	7.6	9.98 (1)		7.69 ± 0.62 (12)
1971					
21 January	19.94	4.8	8.75 (1)	6.69 ± 0.09 (2)	6.62 ± 0.55 (10)
19 February	22.34	0.5		5.18 ± 0.88 (2)	6.16 ± 0.33 (11)
17 March	17.28	6.2			6.30 ± 0.27 (13)
				1969 class	1970 class
13 April	21.22	8.8	6.58 ± 0.53 (5)	6.92 ± 0.23 (3)	
2 June	24.26	19.7	8.91 (1)	6.75 ± 0.37 (10)	5.90 (1)
21 July	21.31	24.1	7.82 ± 0.73 (8)		7.54 ± 0.83 (3)

Table 6 - Seasonal variation of mean serum potassium cocentration in mature, hard blue crabs, Callinectes sapidus, from the York Spit area, Chesapeake Bay, Virginia.

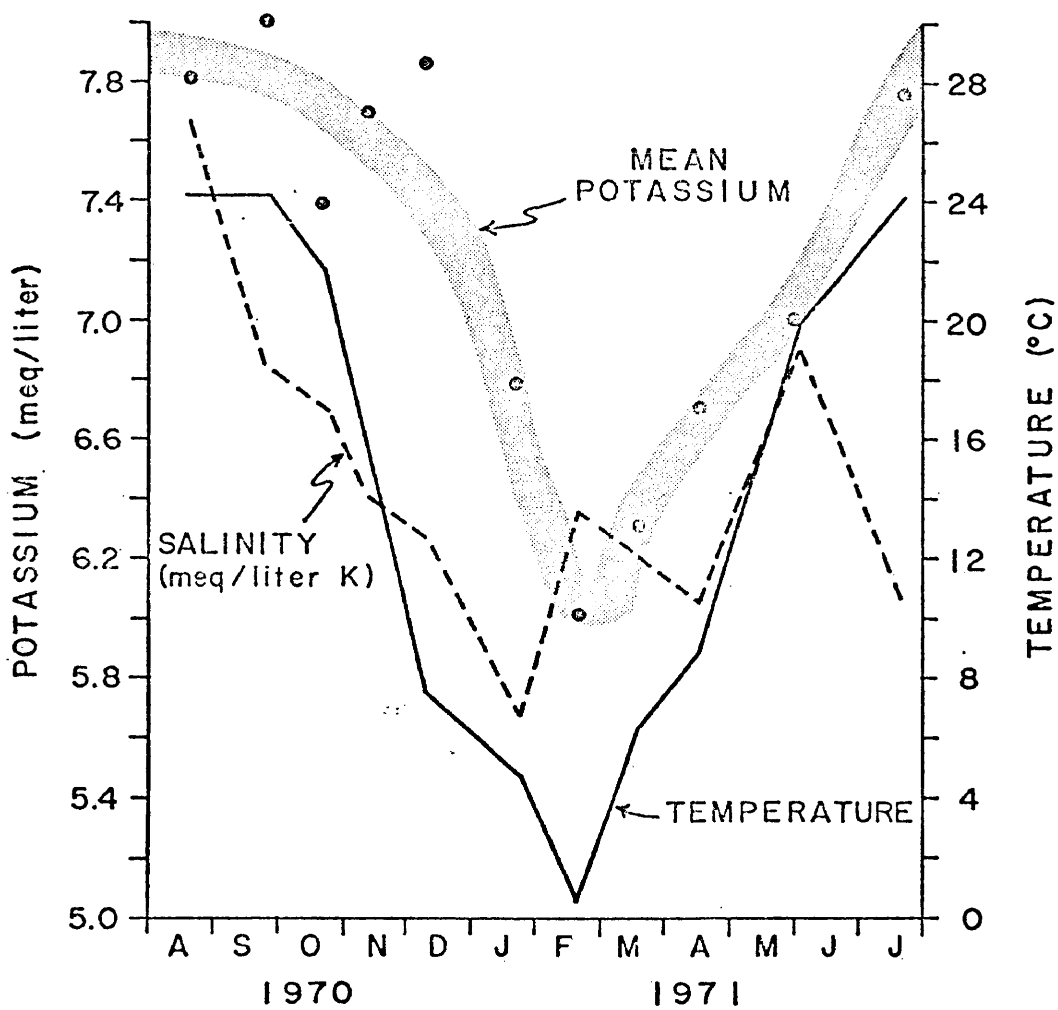

Figure 3 - Seasonal variation of mean serum potassium concentration in mature hard blue crabs, _Callinectes sapidus_, from the York Spit area, Chesapeake Bay, Virginia.

significant sexual or year class differences were found.

In the salinity gradient study, mean serum potassium ranged from 5.41 to 8.35 meq/liter (Table 7). Serum potassium levels are hyperionic below 24 o/oo, isoionic between about 24 - 28 o/oo, and possibly hypoionic above 28 o/oo (Fig. 4). No significant sexual differences were found. A positive correlation (r = .43) was found between serum potassium and salinity.

SERUM CALCIUM

In the seasonal study, mean serum calcium ranged from 23.7 to 34.3 meq/liter (Table 8). No distinct seasonal pattern was evident (Fig. 5). Males had a significantly lower mean serum calcium concentration than females during the month of November, but no other sexual or year class differences were noted.

In the salinity gradient study, mean serum calcium ranged from 25.5 to 37.9 meq/liter (Table 9). Serum calcium was maintained strongly hyperionic over the entire range of salinities in this study (Fig. 6). There was a positive correlation (r = .68) between serum calcium levels and serum total protein levels.

SERUM MAGNESIUM

In the seasonal study, mean serum magnesium ranged from 20.0 to 44.3 meq/liter (Table 10). No distinct seasonal pattern was observed (Fig. 7). Variability appeared to be higher during winter months

Sample date	Area	Salinity (o/oo)	Temperature (°C)	Mean serum potassium ± S.E. (meq/liter) Males (N)	Females (N)
1971					
3 June	YR	14.42	20.7		10.27 (1)
3 June	YR	12.69	20.5	6.76 ± 0.68 (5)	7.65 (1)
3 June	YR	9.05	21.1	7.22 ± 0.53 (4)	8.26 ± 0.91 (2)
3 June	YR	5.09	21.7	5.01 ± 0.41 (4)	5.82 ± 0.62 (4)
7 June	YR	24.26	19.7	6.55 ± 0.25 (4)	6.84 ± 0.22 (3)
7 June	CB	26.34	19.5		6.87 ± 0.48 (3)
8 July	YR	19.60	24.6		7.38 ± 0.09 (3)
8 July	YR	16.72	25.6	6.70 (1)	7.05 ± 0.61 (4)
8 July	YR	13.52	26.5	6.97 ± 0.10 (2)	6.84 ± 0.42 (3)
8 July	PR	6.42	28.2		6.54 ± 0.25 (2)
8 July	PR	0.15	28.3	6.07 (1)	
12 July	CB	20.88	24.1		10.81 (1)
12 July	CB	28.97	21.5	7.47 (1)	8.27 ± 1.41 (2)
4 August	YR	21.63	26.1	7.30 ± 0.64 (4)	7.69 ± 0.35 (2)
4 August	YR	19.90	27.5	5.58 (1)	
4 August	YR	17.40	27.6	7.38 ± 0.51 (4)	6.75 ± 0.40 (4)
4 August	YR	14.29	28.7	6.38 ± 0.32 (4)	7.06 ± 0.57 (4)
4 August	PR	9.30	28.9	7.24 ± 0.86 (4)	
4 August	PR	3.72	29.3	5.77 ± 2.43 (2)	
9 August	YR	21.46	26.5	8.72 ± 0.52 (5)	7.42 ± 0.49 (2)
9 August	CB	24.85	25.7	7.99 ± 0.16 (2)	8.72 ± 0.46 (3)

Area code: YR, York River; CB, Chesapeake Bay; PR, Pamunkey River.
*Significant difference.

Table 7 - Mean serum potassium concentration in mature, hard blue crabs, *Callinectes sapidus*, collected from various salinities in Virginia.

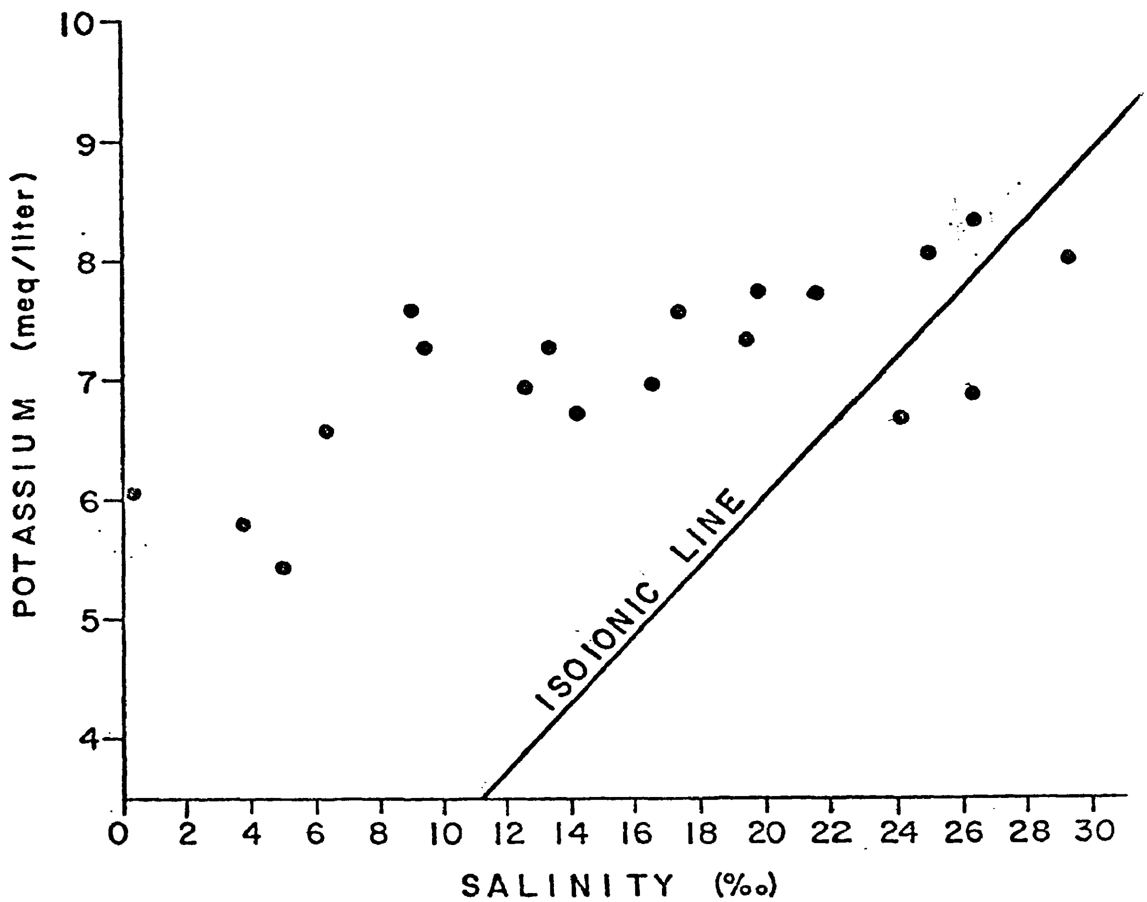

Figure 4 - Mean serum potassium concentration in mature, hard blue crabs, _Callinectes sapidus_, collected from various salinities in Virginia.

Sample date	Salinity (o/oo)	Temperature (°C)	Mean serum calcium ± S.E. (meq/liter)		
			Males (N)	Old females (N)	New females (N)
1970				1968 class	1969 class
18 August	27.01	24.1		32.70 ± 0.02 (2)	26.06 ± 2.06 (11)
23 September	24.06	24.1	24.02 (1)	28.78 ± 3.40 (4)	25.44 ± 1.84 (8)
20 October	23.50	21.6		23.94 ± 5.52 (3)	23.66 ± 1.52 (10)
12 November	22.48	14.9	22.30 ± 2.58 (2)*		33.42 ± 2.00 (11)*
8 December	22.02	7.6	25.46 (1)		30.22 ± 1.38 (12)
1971					
21 January	19.94	4.8	31.32 (1)	24.74 ± 2.52 (2)	26.40 ± 1.34 (10)
19 February	22.34	0.5		36.34 ± 5.14 (2)	32.46 ± 1.50 (11)
17 March	17.28	6.2			34.26 ± 1.32 (13)
				1969 class	1970 class
13 April	21.22	8.8	28.58 ± 2.18 (5)	29.32 ± 2.50 (3)	
2 June	24.26	19.7	24.76 (1)	32.00 ± 2.16 (10)	20.66 (1)
21 July	21.31	24.1	26.38 ± 1.84 (8)		23.70 ± 3.04 (3)

*Significant difference

Table 8 - Seasonal variation of mean serum calcium concentration in mature, hard blue crabs, *Callinectes sapidus*, from the York Spit area, Chesapeake Bay, Virginia.

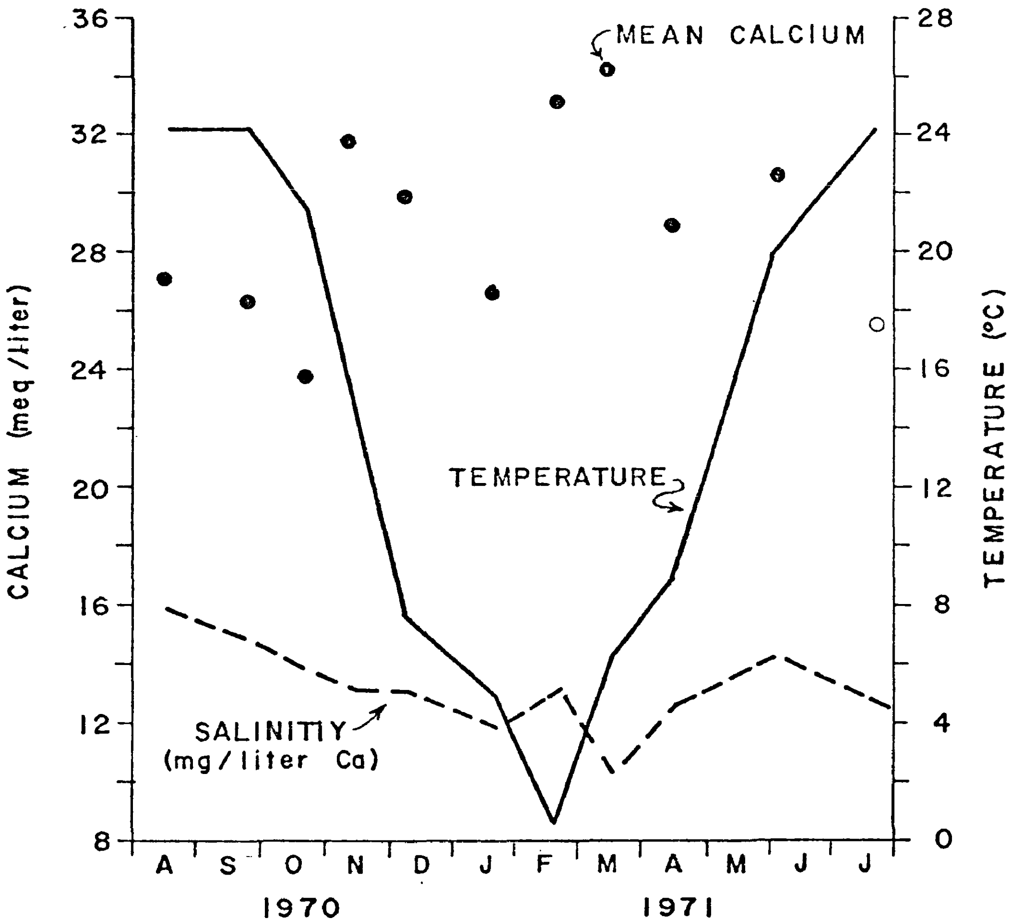

Figure 5 - Seasonal variation of mean serum calcium concentration in mature, hard blue crabs, Callinectes sapidus, from the York Spit area, Chesapeake Bay, Virginia.

Sample date	Area	Salinity (o/oo)	Temperature (°C)	Mean serum calcium ± S.E. (meq/liter)	
				Males (N)	Females (N)
1971					
3 June	YR	14.42	20.7		34.56 (1)
3 June	YR	12.69	20.5	32.84 ± 2.14 (5)	30.04 (1)
3 June	YR	9.05	21.1	29.14 ± 2.02 (4)	26.68 ± 1.75 (2)
3 June	YR	5.09	21.7	29.54 ± 3.07 (4)	28.88 ± 0.71 (4)
7 June	YR	24.26	19.7	31.18 ± 0.64 (4)	32.90 ± 2.39 (3)
7 June	CB	26.34	19.5		28.82 ± 1.64 (3)
8 July	YR	19.60	24.6		25.66 ± 3.64 (3)
8 July	YR	16.72	25.6	21.50 (1)	27.24 ± 0.94 (4)
8 July	YR	13.52	26.5	34.38 ± 2.70 (2)	31.84 ± 0.90 (3)
8 July	PR	6.42	28.2		26.70 ± 3.70 (2)
8 July	PR	0.15	28.3	27.24 (1)	
12 July	CB	20.88	24.1		31.70 (1)
12 July	CB	28.97	21.5	40.82 (1)	36.50 ± 3.56 (2)
4 August	YR	21.63	26.1	28.32 ± 1.69 (4)	27.28 ± 1.25 (2)
4 August	YR	19.90	27.5	20.36 (1)	
4 August	YR	17.40	27.6	26.30 ± 0.40 (4)	25.54 ± 1.43 (4)
4 August	YR	14.29	28.7	30.14 ± 1.82 (4)	28.41 ± 2.41 (4)
4 August	PR	9.30	28.9	29.08 ± 3.72 (4)	
4 August	PR	3.72	29.3	29.96 ± 2.30 (2)	
9 August	YR	21.46	26.5	27.48 ± 2.74 (5)	29.28 ± 2.40 (2)
9 August	CB	24.85	25.7	29.58 ± 1.17 (2)	35.26 ± 1.56 (3)

Area code: YR, York River; CB, Chesapeake Bay; PR, Pamunkey River.
*Significant difference.

Table 9 - Mean serum calcium concentration in mature, hard blue crabs, *Callinectes sapidus*, collected from various salinities in Virginia.

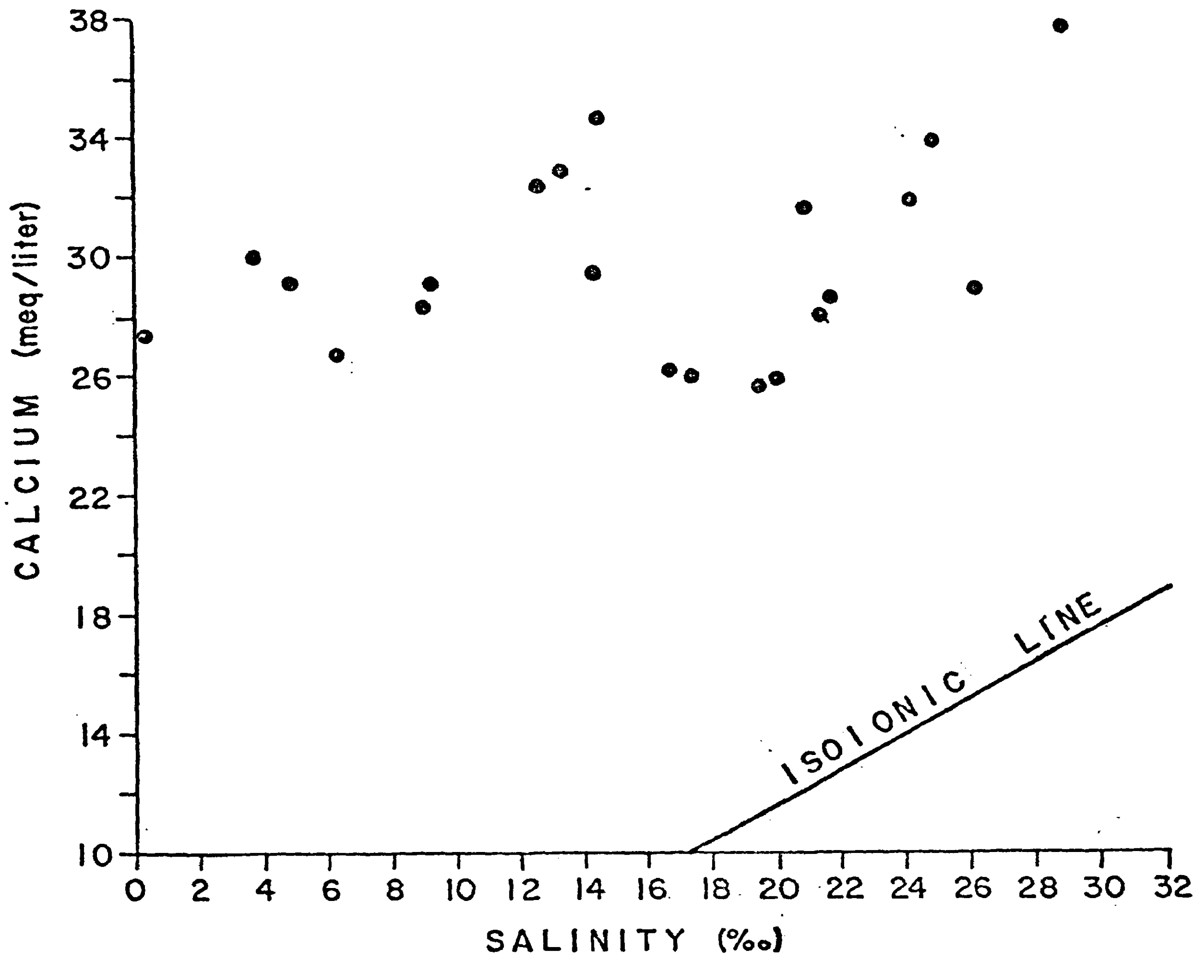

Figure 6 - Mean serum calcium concentration in mature, hard blue crabs, Callinectes sapidus, collected from various salinities in Virginia.

Sample date	Salinity (o/oo)	Temperature (°C)	Mean serum magnesium ± S.E. (meq/liter) Males (N)	Old females (N)	New females (N)
1970				1968 class	1969 class
18 August	27.01	24.1		33.36 ± 1.30 (2)	29.74 ± 1.38 (11)
23 September	24.06	24.1	38.22 (1)	34.28 ± 4.38 (4)*	25.34 ± 1.34 (8)*
20 October	23.50	21.6		33.64 ± 2.12 (3)	31.24 ± 2.02 (10)
12 November	22.48	14.9	28.52 ± 0.30 (2)		31.62 ± 2.18 (11)
8 December	22.02	7.6	36.04 (1)		45.00 ± 1.66 (12)
1971					
21 January	19.94	4.8	25.58 (1)	30.72 ± 0.22 (2)	29.54 ± 1.72 (10)
19 February	22.34	0.5		33.78 ± 6.80 (2)	37.10 ± 2.50 (11)
17 March	17.28	6.2			27.84 ± 1.68 (13)
				1969 class	1970 class
13 April	21.22	8.8	19.06 ± 0.76 (5)	21.62 ± 1.34 (3)	
2 June	24.26	19.7	18.44 (1)	25.20 ± 1.42 (10)	19.88 (1)
21 July	21.31	24.1	25.70 ± 1.96 (8)		26.44 ± 1.64 (3)

*Significant difference

Table 10 - Seasonal variation of mean serum magnesium concentration in mature, hard blue crabs, <u>Callinectes sapidus</u>, from the York Spit area, Chesapeake Bay, Virginia.

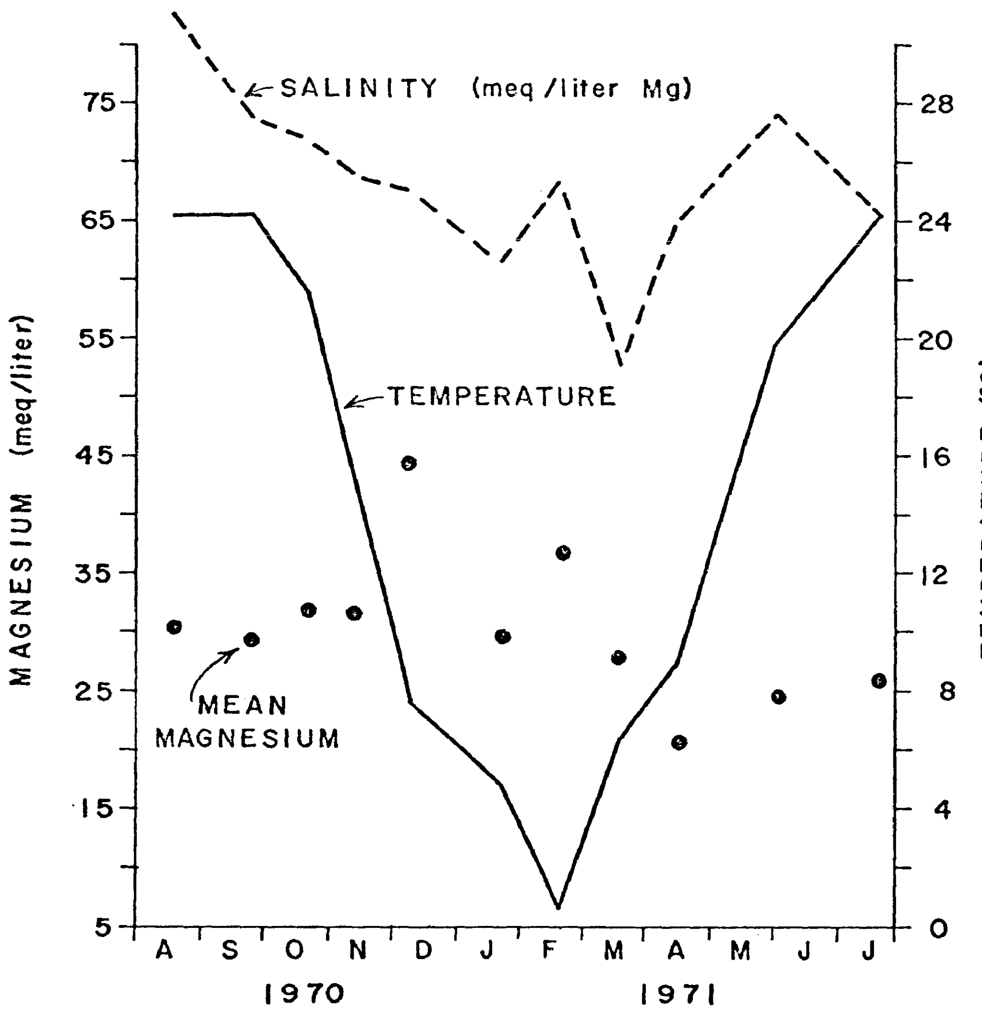

Figure 7 - Seasonal variation of mean serum magnesium concentration in mature, hard blue crabs, *Callinectes sapidus*, from the York Spit area. Chesapeake Bay, Virginia.

(Table 10). There was a significant difference between different year class females for the month of September, but no other significant sex or age linked differences.

In the salinity gradient study, mean serum magnesium ranged from 20.2 to 38.5 meq/liter (Table 11). Serum magnesium is maintained hyperionic between 0 and 5 o/oo, isoionic between about 5 and 10 o/oo, and hypoionic above about 10 o/oo (Fig. 8).

SERUM COPPER AND ZINC

Mean serum copper ranged from 41 to 100 parts per million in the seasonal study (Table 12) and 42 to 107 ppm in the salinity gradient study (Table 13). A significant difference was found between different year class females in September and between males and females in November.

Mean serum zinc ranged from 7.3 to 21.8 ppm in the seasonal study (Table 14) and from 6.2 to 17.4 ppm in the salinity gradient study (Table 15). A significant difference was found between different year class females in August and between males and females in April.

There was no indication of any relationship between serum levels of either of these metals and environmental temperatures or salinities. The relationship between serum levels of these metals and the levels of some of the other serum constituents was, however significant. Serum levels of copper and zinc

Sample date	Area	Salinity (o/oo)	Temperature (°C)	Mean serum magnesium ± S.E. (meq/liter)	
				Males (N)	Females (N)
1971					
3 June	YR	14.42	20.7		35.12 (1)
3 June	YR	12.69	20.5	24.86 ± 1.67 (5)	40.60 (1)
3 June	YR	9.05	21.1	25.44 ± 1.56 (4)	27.98 ± 2.29 (2)
3 June	YR	5.09	21.7	19.14 ± 3.23 (4)	21.34 ± 1.86 (4)
7 June	YR	24.26	19.7	23.88 ± 3.27 (4)	25.84 ± 2.41 (3)
7 June	CB	26.34	19.5		27.54 ± 5.46 (3)
8 July	YR	19.60	24.6		32.88 ± 2.86 (3)
8 July	YR	16.72	25.6	22.58 (1)	22.92 ± 1.58 (4)
8 July	YR	13.52	26.5	25.73 ± 1.95 (2)	24.08 ± 1.07 (3)
8 July	PR	6.42	28.2		28.36 ± 4.22 (2)
8 July	PR	0.15	28.3	20.62 (1)	
12 July	CB	20.88	24.1		25.70 (1)
12 July	CB	28.97	21.5	42.16 (1)	35.10 ± 7.67 (2)
4 August	YR	21.63	26.1	20.66 ± 3.30 (4)	25.12 ± 2.18 (2)
4 August	YR	19.90	27.5	26.50 (1)	
4 August	YR	17.40	27.6	20.52 ± 2.65 (4)	19.36 ± 2.04 (4)
4 August	YR	14.29	28.7	20.44 ± 0.61 (4)	24.94 ± 3.18 (4)
4 August	PR	9.30	28.9	22.68 ± 2.86 (4)	
4 August	PR	3.72	29.3	20.24 ± 3.12 (2)	
9 August	YR	21.46	26.5	25.98 ± 2.99 (5)	31.20 ± 3.13 (2)
9 August	CB	24.85	25.7	27.86 ± 5.69 (2)	29.86 ± 7.45 (3)

Area code: YR, York River; CB, Chesapeake Bay; PR, Pamunkey River.
*Significant difference.

Table 11 - Mean serum magnesium concentration in mature, hard blue crabs, *Callinectes sapidus*, collected from various salinities in Virginia.

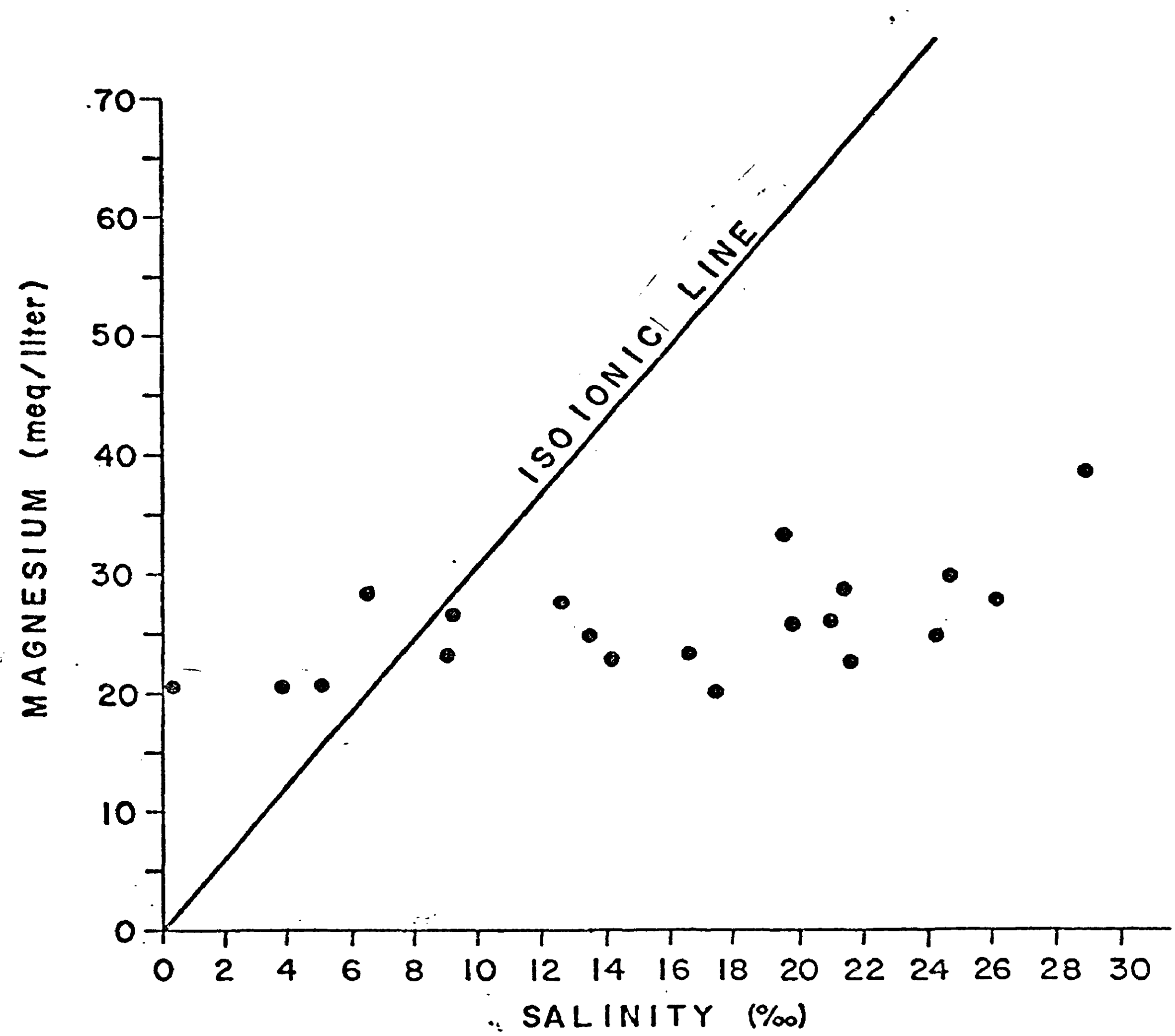

Figure 8 - Mean serum magnesium concentration in mature, hard blue crabs, *Callinectes sapidus*, collected from various salinities in Virginia.

Sample date	Salinity (o/oo)	Temperature (°C)	Mean serum copper ± S.E. (ppm)		
			Males (N)	Old females (N)	New females (N)
1970				1968 class	1969 class
18 August	27.01	24.1		87.1 ± 35.5 (2)	54.7 ± 5.3 (11)
23 September	24.06	24.1	72.9 (1)	82.6 ± 12.2 (4)*	53.2 ± 5.7 (8)*
20 October	23.50	21.6		81.3 ± 27.7 (3)	54.6 ± 3.8 (10)
12 November	22.48	14.9	41.4 ± 4.0 (2)*		93.5 ± 10.7 (11)*
8 December	22.02	7.6	38.8 (1)		65.6 ± 3.4 (12)
1971					
21 January	19.94	4.8	38.5 (1)	101.3 ± 9.5 (2)*	73.9 ± 7.8 (10)*
19 February	22.34	0.5		57.8 ± 14.9 (2)	59.1 ± 4.1 (11)
17 March	17.28	6.2			94.9 ± 11.3 (13)
				1969 class	1970 class
13 April	21.22	8.8	79.7 ± 15.3 (5)	70.7 ± 11.4 (3)	
2 June	24.26	19.7	15.7 (1)	100.2 ± 8.7 (10)	52.2 (1)
21 July	21.31	24.1	72.9 ± 8.1 (8)		84.6 ± 21.4 (3)

*Significant difference

Table 12 - Seasonal variation of mean serum copper concentration in mature, hard blue crabs, <u>Callinectes sapidus</u>, from the York Spit area, Chesapeake Bay, Virginia.

Sample date	Area	Salinity (o/oo)	Temperature (°C)	Mean serum copper ± S.E. (ppm) Males (N)	Mean serum copper ± S.E. (ppm) Females (N)
1971					
3 June	YR	14.42	20.7		150.2 (1)
3 June	YR	12.69	20.5	92.0 ± 24.6 (5)	66.9 (1)
3 June	YR	9.05	21.1	62.7 ± 20.1 (4)	50.3 ± 0.1 (2)
3 June	YR	5.09	21.7	79.7 ± 21.7 (4)	55.6 ± 5.0 (4)
7 June	YR	24.26	19.7	49.5 ± 5.5 (4)	98.1 ± 39.5 (3)
7 June	CB	26.34	19.5		76.9 ± 9.6 (3)
8 July	YR	19.60	24.6		48.5 ± 8.4 (3)
8 July	YR	16.72	25.6	35.3 (1)	60.6 ± 11.3 (4)
8 July	YR	13.52	26.5	85.9 ± 1.6 (2)	79.4 ± 16.2 (3)
8 July	PR	6.42	28.2		71.2 ± 20.6 (2)
8 July	PR	0.15	28.3	81.2 (1)	
12 July	CB	20.88	24.1		95.9 (1)
12 July	CB	28.97	21.5	113.9 (1)	107.0 ± 0.5 (2)
4 August	YR	21.63	26.1	60.4 ± 15.3 (4)	47.7 ± 0.7 (2)
4 August	YR	19.90	27.5	32.6 (1)	
4 August	YR	17.40	27.6	42.1 ± 7.3 (4)	56.8 ± 11.7 (4)
4 August	YR	14.29	28.7	52.9 ± 11.9 (4)	78.4 ± 30.8 (4)
4 August	PR	9.30	28.9	85.9 ± 27.4 (4)	
4 August	PR	3.72	29.3	95.3 ± 22.2 (2)	
9 August	YR	21.46	26.5	59.8 ± 8.4 (5)	76.4 ± 32.2 (2)
9 August	CB	24.85	25.7	69.0 ± 27.9 (2)	93.4 ± 4.0 (3)

Area code: YR, York River; CB, Chesapeake Bay; PR, Pamunkey River.
*Significant difference.

Table 13 - Mean serum copper concentration in mature, hard blue crabs, _Callinectes sapidus_, collected from various salinities in Virginia.

Sample date	Salinity (o/oo)	Temperature (°C)	Mean serum zinc ± S.E. (ppm)		
			Males (N)	Old females (N)	New females (N)
1970				1968 class	1969 class
18 August	27.01	24.1		18.62 ± 8.48 (2)*	7.54 ± 0.67 (11)*
23 September	24.06	24.1	11.74 (1)	10.45 ± 2.42 (4)	6.73 ± 1.10 (8)
20 October	23.50	21.6		12.47 ± 3.16 (3)	8.76 ± 1.32 (10)
12 November	22.48	14.9	9.46 ± 4.86 (2)		14.40 ± 2.08 (11)
8 December	22.02	7.6	20.77 (1)		13.19 ± 1.28 (12)
1971					
21 January	19.94	4.8	14.20 (1)	15.00 ± 4.33 (2)	15.29 ± 2.43 (10)
19 February	22.34	0.5		13.36 ± 0.96 (2)	11.70 ± 0.90 (11)
17 March	17.28	6.2			15.21 ± 1.45 (13)
				1969 class	1970 class
13 April	21.22	8.8	21.81 ± 4.81 (5)*	12.09 ± 2.78 (3)*	
2 June	24.26	19.7	7.31 (1)	14.86 ± 1.33 (10)	9.29 (1)
21 July	21.31	24.1	13.76 ± 3.79 (8)		7.27 ± 2.08 (3)

*Significant difference

Table 14 - Seasonal variation of mean serum zinc concentration in mature, hard blue crabs, *Callinectes sapidus*, from the York Spit area, Chesapeake Bay, Virginia.

Sample date	Area	Salinity (o/oo)	Temperature (°C)	Mean serum zinc ± S.E. (ppm) Males (N)	Females (N)
1971					
3 June	YR	14.42	20.7		20.69 (1)
3 June	YR	12.69	20.5	12.83 ± 2.40 (5)	9.62 (1)
3 June	YR	9.05	21.1	9.68 ± 2.75 (4)	9.75 ± 0.92 (2)
3 June	YR	5.09	21.7	9.90 ± 1.32 (4)	9.23 ± 1.19 (4)
7 June	YR	24.26	19.7	7.92 ± 0.83 (4)	14.37 ± 3.19 (3)
7 June	CB	26.34	19.5		9.91 ± 3.00 (3)
8 July	YR	19.60	24.6		5.65 ± 0.93 (3)
8 July	YR	16.72	25.6	5.48 (1)	6.84 ± 0.48 (4)
8 July	YR	13.52	26.5	10.94 ± 1.94 (2)	9.26 ± 0.75 (3)
8 July	PR	6.42	28.2		8.31 ± 1.79 (2)
8 July	PR	0.15	28.3	8.38 (1)	
12 July	CB	20.88	24.1		14.90 (1)
12 July	CB	28.97	21.5	17.07 (1)	17.38 ± 1.54 (2)
4 August	YR	21.63	26.1	10.19 ± 2.17 (4)	6.19 ± 1.46 (2)
4 August	YR	19.90	27.5	6.49 (1)	
4 August	YR	17.40	27.6	8.57 ± 0.76 (4)	10.64 ± 1.63 (4)
4 August	YR	14.29	28.7	9.61 ± 1.76 (4)	10.04 ± 2.62 (4)
4 August	PR	9.30	28.9	11.00 ± 3.04 (4)	
4 August	PR	3.72	29.3	12.07 ± 2.94 (2)	
9 August	YR	21.46	26.5	7.45 ± 1.15 (5)	10.38 ± 3.27 (2)
9 August	CB	24.85	25.7	11.18 ± 4.34 (2)	12.03 ± 1.99 (3)

Area code: YR, York River; CB, Chesapeake Bay; PR, Pamunkey River.
*Significant difference.

Table 15 - Mean serum zinc concentration in mature, hard blue crabs, *Callinectes sapidus*, collected from various salinities in Virginia.

were found to be correlated (r = .77), but copper was more closely related (r = .91) to total serum protein levels than was zinc (r = .81). Serum levels of these metals are also related to serum calcium levels, with copper showing a considerably weaker relationship (r = .59) than zinc (r = .76). The interrelationships between the various correlation coefficients with the statistical justifications for considering one relationship stronger than another are shown in Table 16.

SERUM CADMIUM AND LEAD

No detectable amounts (0.3 ppm in undiluted serum) of cadmium or lead were found in any serum sample, although about 1/3 of the samples, including at least one sample from each collection, were analyzed for these metals.

Grouping	#	Relation	r	z	$\sqrt{2/(n-3)}$	pairing	t	bar diagram**
Temperature	1	Cl vs temp.	-.92	1.589	.123	1 vs 3	2.39	
	2	Osm vs temp.	-.87	1.344	.123	3 vs 4	3.78*	
	3	Na vs temp.	-.86	1.294	.123			1 2 3 4
	4	K vs temp.	.68	.829	.123			
Salinity	1	Osm vs sal.	.86	1.294	.150	1 vs 3	1.31	
	2	Na vs sal.	.80	1.098	.150	3 vs 4	4.26*	
	3	Cl vs sal.	.80	1.098	.150			1 2 3 4
	4	K vs sal.	.43	.459	.150			
Osm, Na, Cl	1	Osm vs Na	.94	1.738	.094	1 vs 2	3.85*	
	2	Osm vs Cl	.90	1.473	.094	2 vs 3	1.03	
	3	Na vs Cl	.88	1.376	.094			1 2 3
Protein-related constituents	1	Pr vs Cu	.91	1.527	.094	1 vs 2	4.26*	
	2	Pr vs Zn	.81	1.127	.094	2 vs 4	1.39	
	3	Cu vs Zn	.77	1.021	.094	2 vs 5	3.17*	
	4	Ca vs Zn	.76	.996	.094	3 vs 5	2.04	
	5	Pr vs Ca	.68	.829	.094	4 vs 6	3.39*	1 2 3 4 5 6
	6	Ca vs Cu	.59	.677	.094	5 vs 6	1.62	

*Significant 't' (P .05)
**Line indicates no significant difference

Table 16. Results of tests of significant difference between respective correlation coefficients.

DISCUSSION

SERUM SODIUM

Much more work has been done with the regulation of sodium in crustacea than with the other major cations (Krogh, 1939; Beadle, 1957; Robertson, 1960; Lockwood, 1962; Schoffeniels & Gilles, 1970). In each case where the ionic regulation of sodium by a decapod has been studied over a considerable range of salinities, the pattern of sodium regulation has been found to be quite similar to the pattern of osmotic regulation for that animal (Prosser et al., 1955; Gross, 1958; Bryan, 1960; Shaw, 1961; Dehnel, 1967; Bedford, 1972).

The seasonal and salinity-related patterns of serum sodium concentration found in this study are essentially identical to the corresponding patterns of total serum osmotic concentration found by Lynch et al. (1973) for the same animals (including the anomolous low in the Jan. monthly sample). The osmotic concentration data for the 1971 salinity gradient study shows the same pattern of regulation as does sodium, with isotonicity being reached at about 28 - 30 o/oo. However, during the 1970 study, isotonicity was indicated at about 25 o/oo. This small descrepancy is probably due to the effect of temperature; the high salinity stations averaged about 2° C cooler during the 1971 study.

Mantel (1967) also noted a winter rise in serum sodium levels in the blue crab. The adaptive significance of an inverse relationship between temperature and osmotic concentration (and hence sodium and chloride) is not clearly understood, nor is it a universal feature in crustacea (Weber & Spaargaren, 1970; Spaargaren, 1971).

Previously available data on sodium regulation in the blue crab has been very inconclusive. Tan (1962) and Mantel (1967) determined serum sodium levels in blue crabs experimentally subjected to a range of salinities similar to those encountered in this study. The values reported by Tan were more than twice those reported by Mantel at comparable salinities. In both cases water temperatures were maintained at about 20° C. Although the animals used in Tan's study were taken at 5° C and only acclimated for 4 days, the magnitude of the difference in values is too great to be attributed to the effect of temperature. Data from the present study is in virtually exact agreement with the results of Mantel's study.

Gifford (1962) reported serum sodium concentrations in blue crabs subjected to hypersaline environmental conditions. Although salinities he used did not overlap the present study, he observed serum sodium levels which were isoionic at 38 o/oo, it may thus be assumed that isoionic blood sodium levels are maintained in the blue crab between about 25 and 40 o/oo, depending on temperature. Above 40 o/oo Gifford found sodium to be regulated hypoionic to the medium. Therefore, the blue crab can be considered a hyperionic-hypoionic sodium regulator.

Sex differences in osmoregulatory ability were found by Ballard and Abbott (1969), with female blue crabs having significantly higher serum osmoconcentrations than males in lower salinities. Tan (1962) reported a similar sex difference for sodium concentration, with females having substantially higher serum sodium levels than males at 10 and 20 o/oo. At 30 o/oo, however, he found males to have much higher serum sodium levels than females, and the level in females was actually lower than it had been at 20 o/oo.

Although there were not adequate numbers of males and females in any one collection taken for this study to make any statistically sound statements about the presence or absence of sexual differences, there did not seem to be any tendency towards such differences. In general, when any collection contained four or more members of each sex the resultant means for serum sodium were remarkably close. Lynch *et al.*

(1973) suggested that possibly laboratory demonstrated differences are obscured in the field by the mobility of the animals and the variability of environmental conditions in the estuary.

SERUM POTASSIUM

Regulation of serum potassium in decapods has generally been found to follow the same pattern as sodium regulation. Hyperionic regulation occurs at dilute salinities, isoionic conditions occur around full strength seawater, and hypoionic regulation may occur at hypersaline conditions (Gross, 1958; Robertson, 1960; Dehnel, 1967).

While data from the present study is in agreement with the work done on potassium regulation in other crustacea, there is a considerable disparity with the only comparable data for the blue crab. Tan (1962) found potassium to be extremely hyperregulated, with serum concentrations ranging from 15 meq/liter at 10 o/oo to 21 meq/liter at 30 o/oo. Since Gifford (1962), working at hypersaline conditions, did not find concentrations of serum potassium over 15 meq/liter until external concentrations were over 50 o/oo, Tan's data probably is aberrant. Gifford's data fits well with the data from the present study; at 38 o/oo he found a mean serum potassium concentration of about 8.65 meq/liter. By combining the results of the two studies one may state that at summer temperatures the blue crab regulates potassium hyperionically from 0 to about 24 o/oo, isoionically from 24 to 28 o/oo, and hypoionically above that point. Comparing this to the results obtained in the present study for sodium and the results obtained for chloride by Lynch et al. (1973), it would seem that potassium regulation in the blue crab may be more closely related to chloride regulation than sodium regulation, with the former two reaching isotonicity at lower salinities than either sodium or total serum osmotic concentration.

The pattern of seasonal variation of serum potassium found in this study does not appear to be as directly related to temperature as is serum sodium concentration. Previously only Dehnel (1967) had studied seasonal and temperature effects on potassium regulation. He found that winter adapted Hemigrapsus nudus maintained lower serum potassium levels than summer adapted animals, and that this effect was independent of experimental temperature. Quite possibly this seasonal fluctuation might be correlated with the activity of the animals; the blue crab is known to remain relatively dormant during the winter and be particularly active in the summer and fall (Van Engel, 1958). The well known physiological role of potassium in the maintainence of muscle and nerve membrane potentials is compatable with this theory. An active animal may require a considerably more constant and closely regulated level of blood potassium than a dormant one.

SERUM CALCIUM

Because of its role in shell formation, the metabolism of calcium has been more thoroughly studied in decapods than either potassium or magnesium metabolism. However, because it does play a dual role as ion and structural element, the regulation of serum calcium levels is considerably less understood than for the other cations. Calcium complexes with blood protein to a far greater extent than any other serum cation (Robertson, 1960).

Since the calcium levels determined in this study represent total serum calcium, the amount of free calcium present in the blood of the blue crab is somewhat less than the values reported here. The considerable degree to which protein-calcium complexing is occuring may be inferred from the fact that there was a significant correlation between serum calcium and total serum protein levels, but none between serum calcium and external calcium concentrations as indicated by salinity.

The binding properties of protein are structurally dependent; therefore the amount of protein-calcium complexing which occurs in a crustacean serum will depend on the types of protein present as well as the concentrations of the two constituents. Premolt crustaceans are known to undergo sharp rises in the serum levels of both calcium and protein (Florkin, 1960), presumably as calcium is reabsorbed from the exoskeleton and maintained in the bloodstream by means of binding with proteins produced in conjunction with the molt cycle. Although the present study strongly indicates that considerable calcium-protein complexing also occurs in intermolt blue crabs, it is not possible to quantify this effect either in respect to total serum protein or to specific protein fractions from this data. The possibility also remains that the observed correlation is reflection of changes associated with the molt cycle since "intermolt" animals cannot be staged to a precision fine enough to eliminate molt stage as a possible variable.

Because the degree of complexing cannot be quantified from the present data, it is not possible to make assertions about the type of calcium regulation occuring at a given salinity. Hyporegulation, at least at normal salinities, would seem to be highly unlikely in view of the manner in which this ion is conserved during the molt cycle. In general, the results of this study indicate that intermolt blue crabs tend to possess serum calcium of levels of 20-40 meq/liter regardless of salinity or season. This concurs well with Haefner's (1964) determination of a value of about 27 meq/liter for intermolt blue crabs. Gifford (1962) found similar data at high salinities; a range of 19.6 to 43.8 meq/liter and no clear relationship to external salinities.

Because serum calcium levels are related to serum protein levels, the year class differences for serum total protein found by Lynch and Webb (1973) might again be expected for serum calcium. In general they found that each new year class of females exhibited a characteristic seasonal pattern; starting with low values upon their appearance in early summer and then steadily rising until leveling out with the onset of winter.

Again it must be noted that there are inadequate numbers within samples to make any firm statements, but the pattern of variation found for serum protein in females does seem to apply to a certain extent to calcium as well. The 1969 year class had values around the mid-20's in the fall, then rose and stayed around 30 (with the exception of Jan., the month with the anomolous low in osmotic concentration). The 1970 year class appearing the following summer exhibited values in the low 20's. The significant difference between males and females in November was probably not a real difference.

SERUM MAGNESIUM

The regulation of magnesium in crustacea has been studied chiefly in relation to the role of excretion in osmoregulation (Robertson, 1960; Gross &

Marshall, 1960; Gross, 1961; Dehnel, 1967). Although the excretory system of decapods is known to selectively retain potassium, calcium and sometimes sodium, only magnesium is known to be selectively excreted. In general, osmoregulating crustaceans maintain their blood magnesium hypoionic to the environment at all but the most dilute salinities, a condition which is verified for the blue crab by this study. The significance of maintaining low serum magnesium levels has been ascribed to magnesium interferance with neuromuscular transmission (Robertson, 1960) and the effect of the Ca:Mg ratio on tonus and excitability (Robertson, 1953). The degree of magnesium hypotonicity has been shown to be related to the activity of a given species (Lockwood, 1962).

Gifford (1962) reported serum magnesium levels for blue crabs under hypersaline conditions which were considerably higher than would be expected by extrapolation of the present data. This could be a discrepancy or it could mean that magnesium excretion becomes less effective at higher salinities. In any case he does substantiate the conclusion that the blue crab maintains hypoionic blood magnesium levels at all salinities above about 6 to 8 o/oo.

The seasonal variation shown by magnesium during this study seems somewhat obscure, particularly the relatively high December value. Possibly the seemingly greater variation in winter is simply happenstance for this one year.

There did not seem to be any sexual differences indicated for magnesium regulation in the blue crab.

SERUM COPPER AND ZINC

Serum levels of copper and zinc have been found to be extremely well regulated in crustacea (Kerkut, et al., 1961; Bryan, 1964, 1966, 1967; Djangmah, 1970). Excesses are stored in the hepatopancreas or excreted; low environmental concentrations are overcome by the binding of these metals with serum protein, thus maintaining an inward diffusion gradient. Under conditions of normal environmental concentrations, crustaceans maintain serum levels of these metals on the order of 10^3 to 10^4 times greater than that of the environment. Because these metals are being concentrated to such an extent, there is proportionally little unbound zinc or copper in the sera of decapods (Bryan, 1966).

The relationship between serum copper and serum protein shown in Fig. 9 is essentially the same as that found by Horn and Kerr (1963) for the blue crab. Bryan (1968) found very similar ratios between serum copper and blood solid content in 18 other species of decapods. This is not surprising inasmuch as hemocyanins frequently account for most of the organic content of

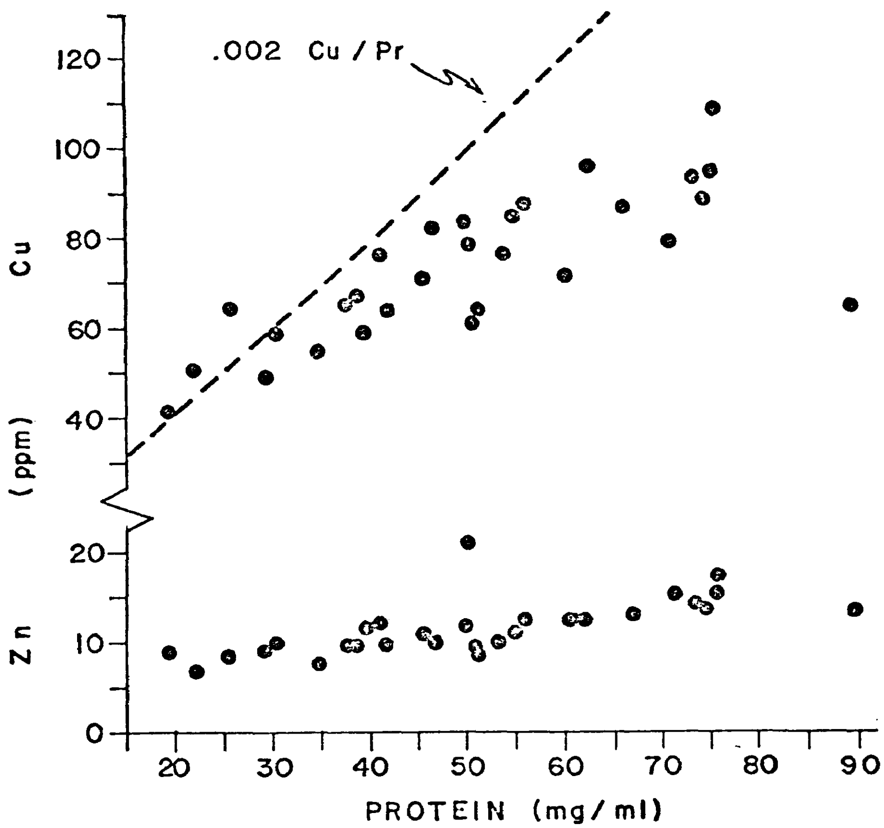

Figure 9 - Relationship of mean serum copper and mean serum zinc to mean total serum protein in mature, hard blue crabs, _Callinectes sapidus_, taken in Virginia waters.

crustacean blood (Manwell & Baker, 1963). Horn and Kerr (1969) found that the copper to protein ratio was useful in predicting the relative amounts of hemocyanin and apohemocyanin, with a copper to protein ratio of .002 representing pure hemocyanin and smaller ratios indicating an apohemocyanin contribution. Those values which fall to the left of the dotted line in Fig. 9 stand in contrast to Horn & Kerr's consideration of .002 as the probable upper limit for the copper/protein ratio. Because this only occurs at low protein concentrations it could indicate that the animals in question were under some kind of stress.

Bryan's extensive work (1964, 1966, 1967, 1968) with zinc regulation in decapods has indicated that there are species specific ratios of zinc to blood solid content. The reason for this is unclear, but likely depends on both the binding properties of the blood proteins and on the efficiency of the mechanisms for removing zinc from the blood. The ratio shown by this study for the blue crab is about intermediate when compared to the other species studied. Working with Austropotamobius pallipes, Bryan (1967) found that serum zinc was chiefly associated with the non-hemocyanin proteins while the converse held true for copper. Horn & Kerr (1969) found that sponge female blue crabs had a lower copper/protein ratio than non-sponge females or males due to a decrease in the percent contribution of hemocyanin to total protein. It would be interesting to know if there was a corresponding increase in the zinc/protein ratio.

Serum zinc and calcium levels were found in this study to be as strongly correlated with each other as they were with total serum protein. This indicates that both may be bound in common to a certain protein or group of proteins. The preceeding discussion suggests that any such protein or proteins are non-hemocyanic. Zinc is a co-factor of carbonic anhydrase, the chief enzyme involved in calcification, but a study by Bryan (1964) indicates that serum levels of carbonic anhydrase and zinc may be only loosely related. He found that a 10 to 30 fold increase in carbonic anhydrase activity was associated with only a 2 to 3 fold increase in zinc concentration in *Homarus vulgaris*. Therefore, while some of the relationship between serum levels of zinc and calcium may be considered to be a result of corresponding metabolic responses to carbonic anhydrase activity, it is probable that common binding with other serum proteins is more important. The correlation between serum levels of copper and calcium is probably not chiefly a result of common complexing with protein but rather a reflection of concomitant increases in the hemocyanin and non-hemocyanin protein fractions as total serum protein increases.

Because of the strong correlations between serum zinc and copper and total serum protein, somewhat the same pattern of seasonal variation is in evidence as was found earlier for protein, with new females having increasingly higher concentrations during the summer and fall and then leveling off.

SERUM CADMIUM AND LEAD

Although the crabs used in this study had no detectable amounts of cadmium or lead in their blood, it would be unwise to attempt a generalization based upon this data. The York River is subject to a minimum of industrial pollution, and environmental concentrations of these metals are also undetectable at the minimum dilution required to conserve the samples for the other analyses (10:1). It may, however, be stated that the blue crab does not concentrate either of these metals under normal conditions. If significant levels of either of these metals are found in blue crab sera, high environmental concentrations might be assumed. Whether high environmental concentrations of cadmium or lead (or other heavy metals) will interfere with zinc and copper metabolism in decapods is an unanswered question.

CONCLUSIONS

The use of the serum constituents considered here for the development of physiological indices for the blue crab seems promising. While further experimental work is needed to establish relationships between various types of environmental stress and serum levels of the constituents in question, certain possibilities are suggested by the present study.

Lynch (1972) noted depressed osmotic concentrations in laboratory-held blue crabs and increased osmotic concentrations in thermally stressed animals (in contrast to seasonally observed variations). Determination of the relative contributions of chloride and the various cations to these changes could prove to be of predictive value. Serum levels of potassium and magnesium could prove to have independent value. If serum potassium levels are indeed linked to the activity level of blue crabs, it might be expected that certain types of stress might result in decreased or increased concentrations of that ion. Isotonic levels of magnesium at any salinity above about 10 o/oo would almost undoubtedly indicate some form of excretory failure.

The possibilities for the use of serum copper and zinc levels in physiological indices seem numerous.

Horn and Kerr (1963, 1969) have already suggested that copper and hemocyanin levels are extremely closely related in the blue crab. If Bryan's (1964) finding that zinc is more closely associated with non-hemocyanin proteins holds true for the blue crab, an index using both these metals could be developed to predict both total protein and the relative contribution of hemocyanin. Even should this not prove feasible, the use of either or both of these metals in indices with total serum protein might prove valuable.

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VITA

James Alden Colvocoresses

Born in Superior, Arizona, 5 March 1950. Graduated W. T. Woodson High School, Fairfax, Virginia, June 1967. Received B.S. (Biology) Tulane University, New Orleans, Louisiana, May 1971. Graduate Research Assistant, Virginia Institute of Marine Science, February 1972 to date.